"Cyanogenesis in Plants. Part III.—On Phaseolunatin, the Cyanogenetic Glucoside of *Phaseolus lunatus*." By WYNDHAM R. DUNSTAN, M.A., F.R.S., Director of the Imperial Institute, South Kensington, and THOMAS A. HENRY, D.Sc., (Lond.). Received June 10,—Read June 18, 1903.

(From the Scientific Department of the Imperial Institute.)

Phaseolus lunatus is an annual plant, probably indigenous to South America, but now generally cultivated throughout the tropics, where its edible bean is used as a vegetable. The plant presents much the same appearance as the common French bean, but the flowers are smaller and more numerous, whilst the pods are crescent-shaped and contain only two or three seeds. The latter, according to Cordemoy,* are violet in the wild state, light brown with violet hues or patches when semi-cultivated, and white in the cultivated state. The beans produced by the wild plant are known in Mauritius as *Pois d'Achery*, those from the semi-cultivated plant as *Pois Amer*, whilst the cultivated product is termed *Pois Adam* or *Pois Portal*, and in English-speaking colonies Lima or Duffin beans.

Whilst the white cultivated beans of *Phaseolus lunatus* have never been known to be poisonous, the coloured beans as well as the plant itself in the semi-wild state have frequently exhibited markedly poisonous properties, and attention is directed to this difference between the white and coloured seeds by Church.†

The semi-cultivated plant grown in Mauritius, where it is utilised as green manure and occasionally as cattle fodder, was examined in 1900 by M. Bonamé, Director of the Agricultural Station at Mauritius, in order to ascertain the cause of its poisonous action. The beans were shown to furnish hydrocyanic acid when crushed and moistened with water.‡ The hydrocyanic acid was found not to exist as such in the plant, but to be in some state of combination, probably in the form of a glucoside which, owing to the simultaneous occurrence in the cells of the plant of a hydrolytic enzyme, underwent hydrolysis, furnishing hydrocyanic acid as one product. No attempt, however, was made by M. Bonamé to isolate the glucoside or enzyme, and only indirect evidence of their existence was recorded on the analogy of the bitter almond. Prussic acid was found to be produced by all parts of the plant, though in greatest quantity by the seeds.

The fresh plant was examined later by van Romburgh,§ who showed

* 'Flore de la Réunion,' 1895.

† 'Food-grains of India,' p. 155.

‡ 'Rapport Annuel de la Station Agronomique,' 1900, p. 94.

§ 'Annales du Jardin Botanique de Buitenzorg,' Series II, vol. 1, p. 2.

that when crushed, moistened with water and distilled, it furnished a distillate containing hydrocyanic acid and acetone, and this author drew attention to the fact that he had observed the simultaneous production of acetone and hydrocyanic acid in several other plants, notably in *Manihot utilissima*, which furnishes the cassava of the West Indies.

The observations of Bonamé and of van Romburgh suggested to us the advisability of examining *Pois d'Achery* in continuation of the investigation of the chemical origin of the prussic acid of plants, on which we have been engaged for some time. For the material, which is somewhat difficult to procure, we are indebted to M. Bonamé, who, at the instance of the Colonial Office, collected in Mauritius and forwarded to us two large samples of the beans.

Preliminary Observations.

When a few of the beans are powdered and moistened with cold water, the odour of hydrocyanic acid becomes perceptible in a few minutes. If boiling water is used and the vessel is immediately closed and allowed to cool, no odour of prussic acid is perceptible, and no evidence of its production can be obtained by the application of the usual test to distillates from such preparations. These observations confirm those recorded by Bonamé,* and indicate that the production of hydrocyanic acid is connected with the action of an enzyme.

Estimation of the Amount of Hydrocyanic Acid Produced.

It was found to be impossible to estimate the amount of hydrocyanic acid, obtainable from a weighed quantity of the powdered beans, by soaking the powder in water and subsequent distillation, owing to the continuous frothing over of the liquid. This difficulty, which was experienced by previous workers, was avoided by extracting in a Soxhlet percolator a weighed quantity of the finely ground seeds with 90 per cent. alcohol, distilling off the solvent, hydrolysing the glucoside contained in the residue by distilling with dilute sulphuric acid until hydrocyanic acid no longer appeared in the distillate, and then estimating, by one of the usual methods, the amount of prussic acid thus produced.

Owing to the production of some volatile reducing substance, which was carried over with the hydrocyanic acid in this process, it was difficult to titrate the acid volumetrically by Liebig's method, which we have employed in previous cases, since the end reaction was obscured by the formation of a slight precipitate of reduced silver.

* *Loc. cit.*

A modification of the method used by Fordos and Gelis* has, therefore, been used in the present instance. This consists in adding volumetric iodine solution to the liquid containing hydrocyanic acid until the whole of the latter has been converted into cyanogen iodide according to the equation:

$$HCN + I_2 = HI + CNI.$$

The formation of cyanogen iodide is, however, quickly inhibited by the accumulation in the liquid of hydriodic acid. The originators of the process attempted to overcome this difficulty by first rendering alkaline with potash the liquid to be titrated, and then adding water aërated with carbon dioxide to convert any excess of alkali into the acid carbonate. We have found, however, that it is easier to titrate the hydrocyanic acid with iodine in presence of sodium hydrogen carbonate than to titrate the alkali cyanide under these conditions, and consequently we have modified the process by adding to the liquid to be titrated excess of sodium hydrogen carbonate. As this method of titration has rarely been used previously, we have confirmed the results so obtained by gravimetric estimations of the amount of hydrocyanic acid in the distillate.

Colour of beans.	Hydrocyanic acid produced, estimated by the modified Fordos and Gelis method, per cent.	Estimated as silver cyanide.
Dark brown.....	0·0872	—
	0·0955	0·1020
Purple	0·088	—
	0·088	—
Light brown	0·041	0·0503

It thus appears that the beans with dark brown or purple markings furnish more prussic acid than the beans with light brown markings, and that the largest amount produced was equivalent to about one-tenth per cent. calculated on the dry beans.

Isolation and Determination of the Constitution of the Glucoside Phaseolunatin.

The finely powdered beans were exhausted by cold percolation with purified methylated alcohol. The mixed alcoholic liquors were then concentrated to a syrup, which was boiled repeatedly with water in order to separate the glucoside from resin. Tannin, gum and extractive matters were removed form the solution by precipitation with lead acetate, the excess of the latter being subsequently eliminated as lead

* 'Journ. de Chimie et de Pharmacie,' vol. 23, p. 48.

sulphide. The colourless liquid so obtained was evaporated in a vacuous desiccator, the syrupy residue being well stirred every day, until it solidified to a mass of colourless acicular crystals, which were freed from the viscous mother liquor by absorption of the latter in a porous tile. The glucoside was purified by recrystallisation from water. It readily forms super-saturated solutions with this solvent, from which it separates only after vigorous stirring. The glucoside is almost insoluble in absolute alcohol, ether and petroleum, but is slightly soluble in acetone, chloroform and ethyl acetate; it also dissolves in alcohol containing water, but does not readily crystallise from such solutions even when concentrated. The glucoside crystallises in spreading rosettes of colourless needles from $\frac{1}{2}$ to 1 inch in length, which melt at 141° C.

Combustions of specially purified material dried at 100° C. until of constant weight gave the following results :—

0·2277 gramme gave	0·4047 gramme	CO_2 =	48·3 per cent.	C.
	0·1412 „	H_2O =	6·8 „	H.
0·1537 gramme gave	0·2710 „	CO_2 =	48·08 „	C.
	0·0972 „	H_2O =	7·02 „	H.

The formula $C_{10}H_{17}O_6N$ requires C 48·1 per cent.; H 6·8 per cent.

The correctness of this formula has been confirmed by estimations of the dextrose produced on hydrolysis. To this glucoside we propose to assign the name—Phaseolunatin.

Specific Rotation of Phaseolunatin.

This constant was determined by observing the rotations produced by a 2·7608 per cent. solution in water contained in a 20 cm. tube. The mean of ten readings was :—

$$-1° 27',$$

whence the specific rotation is

$$[\alpha]_D = -26°·2.$$

Action of Acetic Anhydride on Phaseolunatin.

About 0·5 gramme of the glucoside was dissolved in 5 c.c. of acetic anhydrine, the solution warmed on the water bath for 2 hours, then poured into alcohol and the mixture warmed until the oily precipitate first formed became hard and granular. After filtering, the precipitate was dissolved in water, the solution decolorised with animal charcoal and evaporated to a syrup in a vacuous desiccator. The sticky residue could not be crystallised; it was neutral in reaction, gave ethyl acetate on hydrolysis with concentrated sulphuric acid in presence of alcohol, and was probably, therefore, an acetyl derivative of the glucoside.

Hydrolysis of Phaseolunatin by Acids.

The hydrolysis was usually effected by heating the glucoside in aqueous solution with dilute hydrochloric acid at 100° C under a reflux condenser. The resulting solution had a strong odour of hydrocyanic acid and afforded a distillate giving the usual reactions of this substance. This distillate also gave a precipitate of iodoform on the addition of an alkali hydroxide followed by solution of iodine. The hydrocyanic acid was removed by conversion into Prussian blue, which was filtered off, the filtrate made slightly alkaline and distilled until the iodoform reaction was no longer obtained. To the distillate, hydroxylamine hydrochloride and a slight excess of sodium carbonate were added and the mixture after standing 2 hours shaken with ether. The ethereal liquid was separated, dried thoroughly over calcium chloride and allowed to spontaneously evaporate. A colourless crystalline substance then remained which melted at 58°, and had the characteristic odour of *acetoxime*. The latter is, however, not a satisfactory derivative for the identification of such small quantities of acetone as were present, and recourse was therefore had to the preparation of dibenzylidene acetone. To some of the purified distillate freed from hydrocyanic acid in the manner indicated were added a few drops of a 10-per-cent. aqueous solution of potassium hydroxide, followed by a similar quantity of benzaldehyde and sufficient ethyl alcohol (free from acetone) to produce a clear solution. This mixture on standing became cloudy, and in a few hours deposited a crop of golden yellow lamellæ which melted at 112° and exhibited all the properties of *dibenzylidene acetone* (m.p. 112°).

The remainder of the distillate was neutralised and evaporated to a small volume, decolorised with animal charcoal and examined in a polarimeter. The observed rotation was dextrorotatory, but the dark colour of the solution precluded a quantitative determination of its amount. When heated with a little phenylhydrazine at 100° C. this liquid afforded an osazone, which when recrystallised from alcohol melted at 206° (phenylglucosazone melts at 206°).

Estimation of Sugar produced by Acid Hydrolysis.

0·1746 gramme of the glucoside was dissolved in 100 c.c. of water. Portions of this solution were heated with dilute hydrochloric acid at 100° for varying periods. It was found that under these conditions about $2\frac{1}{2}$ hours were required for complete hydrolysis.

Quantity of glucoside used.	Time of heating.	Dextrose found.	Dextrose produced, per cent.
0·0524	2 hours	0·0699 × 0·5042 gramme	67·3
0·0524	2 ,,	0·0714 × 0·5042 ,,	68·7
0·0698	2½ ,,	0·0975 × 0·5042 ,,	70·4
0·1309	3 ,,	0·1676 × 0·5042 ,,	64·6

The equation

$$\underset{\text{Phaseolunatin.}}{C_{10}H_{17}O_6N} + H_2O = \underset{\text{Dextrose.}}{C_6H_{12}O_6} + \underset{\text{Acetone.}}{(CH_3)_2CO} + \underset{\text{Hydrocyanic acid.}}{HCN}.$$

requires the production of 72·5 per cent. of dextrose.

Alkaline Hydrolysis of Phaseolunatin. Phaseolunatinic Acid.

When the glucoside is heated in aqueous solution with solutions of potassium, sodium or barium hydroxides, it undergoes hydrolysis, with the production of ammonia and the formation of a new acid glucoside, phaseolunatinic acid. This, like most compounds of the same class, *e.g.*, amygdalinic, lotusinic and dhurrinic acids, is an amorphous gum-like substance, furnishing gum-like salts which are soluble in water, but insoluble in absolute alcohol. The sodium salt may be obtained pure by adding phaseolunatin to a solution of sodium ethoxide in alcohol. The glucoside at first dissolves, but almost immediately afterwards there is formed an amorphous white precipitate of the sodium salt. This retains a pulverulent form so long as it remains in the dry alcohol, but on removal from the liquid by filtration it begins to absorb water and becomes gum-like. The constitution of this acid glucoside was arrived at by an investigation of the products obtained from its hydrolysis by acids.

About 2 grammes of the original glucoside was heated at 100° C. for two hours with excess of baryta water. On evaporation there remained a varnish-like residue of the barium salt of the acid glucoside. To this excess of dilute sulphuric acid was added, the precipitated barium sulphate removed by filtration and the filtrate boiled for 15 minutes under a reflux condenser. The liquid was cooled, extracted with ether, the ethereal solution dried over calcium chloride and the solvent distilled off, when there remained an oil of an unpleasant rancid odour. This was boiled with water, the aqueous solution after filtration neutralised with baryta water and evaporated to a small volume, when minute colourless glistening plates of a barium salt separated. This on analysis gave the following result:—

0·1569 gramme gave 0·1077 $BaSO_4$ = 68·73 per cent. $BaSO_4$.

Barium α-hydroxy*iso*butyrate, $[(CH_3)_2C(OH)COO]_2Ba$, requires 68·01 per cent. $BaSO_4$.

The residual liquid left after extraction with ether reduced Fehling's solution, and when warmed with phenylhydrazine gave a crystalline precipitate of phenylglucosazone, melting at 206°

Phaseolunatinic acid is thus proved to be the dextrose ether of α hydroxyisobutyric acid.

The alkaline hydrolysis of phaseolunatin therefore takes place according to the following equation:—

$$\begin{matrix}CH_3\\CH_3\end{matrix}\!>\!C(CN).O.C_6H_{11}O_5 + 2H_2O = \begin{matrix}CH_3\\CH_3\end{matrix}\!>\!C(COOH).O.C_6H_{11}O_5 + NH_3.$$

Dextrose ether of acetone cyanhydrin (phaseolunatin).

Dextrose ether of α-hydroxy*iso*butyric acid (phaseolunatinic acid).

The acid glucoside thus formed then undergoes hydrolysis by acids in the following manner:—

$$\begin{matrix}CH_3\\CH_3\end{matrix}\!>\!C(COOH).O.C_6H_{11}O_5 + H_2O = \begin{matrix}CH_3\\CH_3\end{matrix}\!>\!C(COOH).OH + C_6H_{12}O_6.$$

Dextrose ether of α-hydroxy*iso*butyric acid.

α-Hydroxy*iso*butyric acid. Dextrose.

It is therefore proved that phaseolunatin is the dextrose ether of acetone cyanhydrin.

The Enzyme of Phaseolus lunatus.

The hydrolytic enzyme of *Phaseolus lunatus* was isolated in the usual manner by pouring an aqueous extract of the plant (containing chloroform as a preservative) into excess of alcohol and collecting the precipitated proteid matter. This precipitate was then re-dissolved in water and re-precipitated by alcohol. Prepared in this way the enzyme is an amorphous white powder almost completely soluble in water; it readily hydrolyses amygdalin, salicin, and phaseolunatin. The latter is also hydrolysed by the emulsin of sweet almonds, so that it is probable that the enzyme of *Phaseolus lunatus* is emulsin, although in the present state of our knowledge of the composition and reactions of enzymes it is impossible to prove with certainty the identity of two enzymes of different origin.

In the two previous papers of this series,* it has been pointed out that the presence of cyanogenetic glucosides in *Lotus arabicus* and *Sorghum vulgare* is confined to those parts of the plant in which metabolism is actively proceeding, and that the glucoside no longer occurs when the plant attains maturity, and is not present in the seeds. In the case of *Sorghum vulgare*, cultivation does not appear to diminish the production of the glucoside. *Phaseolus lunatus* presents a different group of facts since, as M. Bonamé has shown, the mature semi-

* 'Phil. Trans.,' B, vol. 194, 1901, p. 515, and A, vol. 190, 1902, p. 399.

cultivated plant furnishes prussic acid, and, as is proved in the present communication, the seeds of the wild Mauritius plant contain considerable quantities of the cyanogenetic glucoside phaseolunatin, which, however, is not found in the seeds of the same plant after systematic cultivation.

Rangoon Beans.

Whilst the investigation of the glucoside of *Phaseolus lunatus* was in progress, there were received at the Imperial Institute several specimens of beans imported into this country from Burma for the manufacture of a feeding stuff for cattle under the names of "Rangoon," "Burma," or "Paigya" beans. These beans varied in colour from light to dark brown with purple patches, and closely resembled the seeds of *Phaseolus lunatus* both in appearance and size, and are, no doubt, derived from this plant. On examination in the manner previously described, Rangoon beans yielded small quantities of hydrocyanic acid, amounting usually to not more than 0·004 per cent. The isolation of the small quantity of glucoside represented by such a proportion of hydrocyanic acid was not possible, but evidence of the existence of the glucoside phaseolunatin was obtained by extracting several pounds of the beans and hydrolysing the extract with dilute hydrochloric acid, when a distillate containing both hydrocyanic acid and acetone was obtained, the latter being identified by its conversion into dibenzylidene acetone.

General Considerations.

The present investigation had for its principal object the determination of the question as to whether the production of prussic acid in the seeds of *Phaseolus lunatus* originated with a glucoside, and if so to isolate this constituent and ascertain its chemical composition. Phaseolunatin proves to be a cyanogenetic glucoside with an aliphatic nucleus, and in this respect differs from the glucosides of this class already known, viz., amygdalin, lotusin and dhurrin, which contain aromatic (benzenoid) nuclei.

The occurrence in *Phaseolus lunatus*, apparently throughout its life history, of a cyanogenetic glucoside, together with the enzyme appropriate for its hydrolysis, seems to strengthen the view expressed by us in a previous paper, that these glucosides must play some definite part in the metabolism of plants.

Treub, as the result of his investigations of the production and distribution of hydrocyanic acid in *Pangium edule*, suggested that the immediate precursor (probably a cyanogenetic glucoside) of the acid in this plant is a formative material utilised in the synthesis of proteid. In this connection it is of interest to note the ease with which cyanogen

compounds of this type can, by processes of reduction, be converted into amino-derivatives, which recent researches indicate as the materials from which, by processes of condensation, proteids originate.

his supposition implies that cyanogenetic glucosides are to be regarded as plastic materials, whilst the enzymes with which they are associated must be considered as aplastic substances with the definite function of decomposing and possibly also producing cyanogenetic glucosides, since the hydrolytic action of enzymes appears to be reversible.

This suggestion may explain the occurrence of cyanogenetic glucosides in *Lotus arabicus* and *Sorghum vulgare* during that period of their life-history in which metabolism is active and their disappearance when the plants have matured and produced seeds, since this period coincides with that in which the synthesis of proteid in the plant is actively proceeding. Although *Phaseolus lunatus* resembles *Lotus arabicus* and *Sorghum vulgare* in containing a cyanogenetic glucoside, it differs from these plants in continuously secreting this glucoside which is likewise found in the seeds. In this respect Phaseolus resembles the bitter almond. The seed produced by *Phaseolus lunatus* under cultivation, however, does not contain phaseolunatin, just as the seed of the sweet almond, which there is reason to believe is produced by the cultivation of *Prunus amygdalus*, contains no amygdalin. It is impossible without further knowledge of the causes which influence plants in the selection of reserve materials to offer any explanation of the fact that these glucosides appear as reserve materials in the seeds of *Phaseolus lunatus* and in those of the bitter almond, but not in those of *Lotus arabicus* and *Sorghum vulgare*.

The reason for the disappearance of cyanogenetic glucosides from the seeds of *Phaseolus lunatus* and the bitter almond when cultivated, is probably to be found in the stimulus to metabolism resulting from improved nutrition and environment. These, as is well known, lead to the more rapid utilisation of plastic substances, with the result that there is very little, or possibly none, of the cyanogenetic glucoside available for storage as reserve material in the seeds of the cultivated plant. The enzymes on the other hand are aplastic substances performing definite synthetical and analytical functions without themselves undergoing change, and consequently it is to be expected that they would be found alike in the seeds of the wild and of the cultivated plants. The enzyme emulsin occurs in the seeds of the cultivated *Phaseolus lunatus* as well as in those of the sweet almond, although the cyanogenetic glucoside has disappeared under the influence of cultivation.

The observations recorded by the authors in a previous paper,* with regard to the existence of the cyanogenetic glucoside dhurrin in

* Part II, *loc. cit.*

Sorghum vulgare and the consequent production of prussic acid by this plant, have led to the examination by J. C. Brunnich,* Chemist to the Agricultural Department, Brisbane, of the varieties of this plant grown in Queensland, which have been long known to be poisonous to cattle under certain conditions, although the nature and origin of the poison had not been discovered. Brunnich has now determined the amounts of hydrocyanic acid produced when weighed quantities of the plants grown under different conditions are crushed with water. The results thus obtained confirm those already recorded by the authors in the case of sorghum grown in Egypt, and show that the amount of cyanogenetic glucoside contained in the stem and leaves of the plant increases until the seeds are ripe, after which it rapidly diminishes until the glucoside finally disappears. Brunnich finds that cultivation of sorghum on land heavily manured with sodium nitrate leads to an increased production of the cyanogenetic glucoside in the stem and leaves.

"The Differential Invariants of Space." By Professor A. R. FORSYTH, Sc.D., LL.D., F.R.S. Received June 18,—Read June 18, 1903.

(Abstract.)

The memoir is devoted to the consideration of the differential invariants of ordinary space and of a surface or surfaces in that space; they are the functions of the fundamental magnitudes of space and of quantities connected with the surface or surfaces which remain unaltered in value through all changes of the independent variables of position.

The method used arises through the obviously natural development of the method used for the corresponding investigations concerned with a surface and with curves upon the surface, which formed the subject of an earlier memoir by the author. The partial differential equations, characteristic of the invariance, are formed, and then the most general solution of these equations is constructed. At a certain stage in the latter process, the equations then remaining unsolved can be transformed, so that they become the invariants and the contravariants of a set of simultaneous ternary forms. The results of the latter theory are then used to complete the solution of the equations.

The main part of the memoir is devoted to obtaining the invariants; and the explicit expressions of the invariants, up to the third order inclusive as associated with a single surface, are given. Further,

* 'Trans. Chem. Soc.,' 1903.